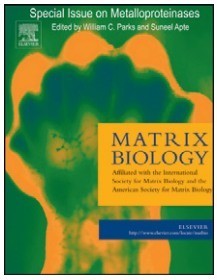

ADAMTS proteases and cancer

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Abstract

ADAMTSs (A disintegrin and metalloprotease domains with thrombospondins motifs) are complex extracellular proteases that have been related to both oncogenic and tumor-protective functions. These enzymes can be secreted by cancer and stromal cells and may contribute to modify the tumor microenvironment by multiple mechanisms. Thus, ADAMTSs can cleave or interact with a wide range of extracellular matrix components or regulatory factors, and therefore affect cell adhesion, migration, proliferation and angiogenesis. The balance of protumor versus antitumor effects of ADAMTSs may depend on the nature of their substrates or interacting-partners upon secretion from the cell. Moreover, different *ADAMTS* genes have been found overexpressed, mutated or epigenetically silenced in tumors from different origins, suggesting the direct impact of these metalloproteases in cancer development. However, despite the important advances on the tumor biology of ADAMTSs in recent years, more mechanistic and functional studies are necessary to fully understand how these proteases can influence tumor microenvironment to potentiate cancer growth or to induce tumor regression. This review outlines current and emerging connections between ADAMTSs and cancer.

Introduction

In 1997, Kuno et al. reported the identification and cloning of a new ADAM (*A D*isintegrin *A*nd *M*etalloproteinase domains) metalloprotease in a cell model of colon cancer cachexia [1]. This enzyme was named ADAMTS-1 (*A D*isintegrin *A*nd *M*etalloproteinase with *T*hrombo*S*pondin motifs) to remark the presence of three thrombospondin type-1 (TS1) motifs within its structure. The first TS1 motif was located in the middle region of the amino acid sequence of this enzyme, and the remaining two were situated in tandem at the carboxy-terminal region of the protein. Another important difference with respect to the classical ADAMs was the finding that ADAMTS-1 was a secreted enzyme lacking the transmembrane domain placed at the carboxy-terminal end of the membrane-anchored ADAMs. Both ADAMs and the closely related MMPs were metalloproteases largely associated with tumorigenesis [2], therefore it was proposed that ADAMTS-1 could also influence tumor growth through modulation of cell proliferation, migration, inflammation and angiogenesis processes.

The discovery of ADAMTS-1 encouraged the search for new *ADAMTS* genes. The availability of the first draft of the human genome sequence and the efforts of different laboratories allowed to complete the molecular cloning of the 19 human ADAMTSs in 2003 [3,4]. Each newly identified ADAMTS displayed a domain organization similar to that shown by ADAMTS-1, characterized by the presence of a central TS1 motif and a variable number of TS1 repeats at the carboxy-terminal region, as well as the absence of a transmembrane domain. In addition, other ancillary domains described in ADAMTSs but not in ADAMs were the spacer region, located after a cysteine-rich region in all ADAMTSs; a

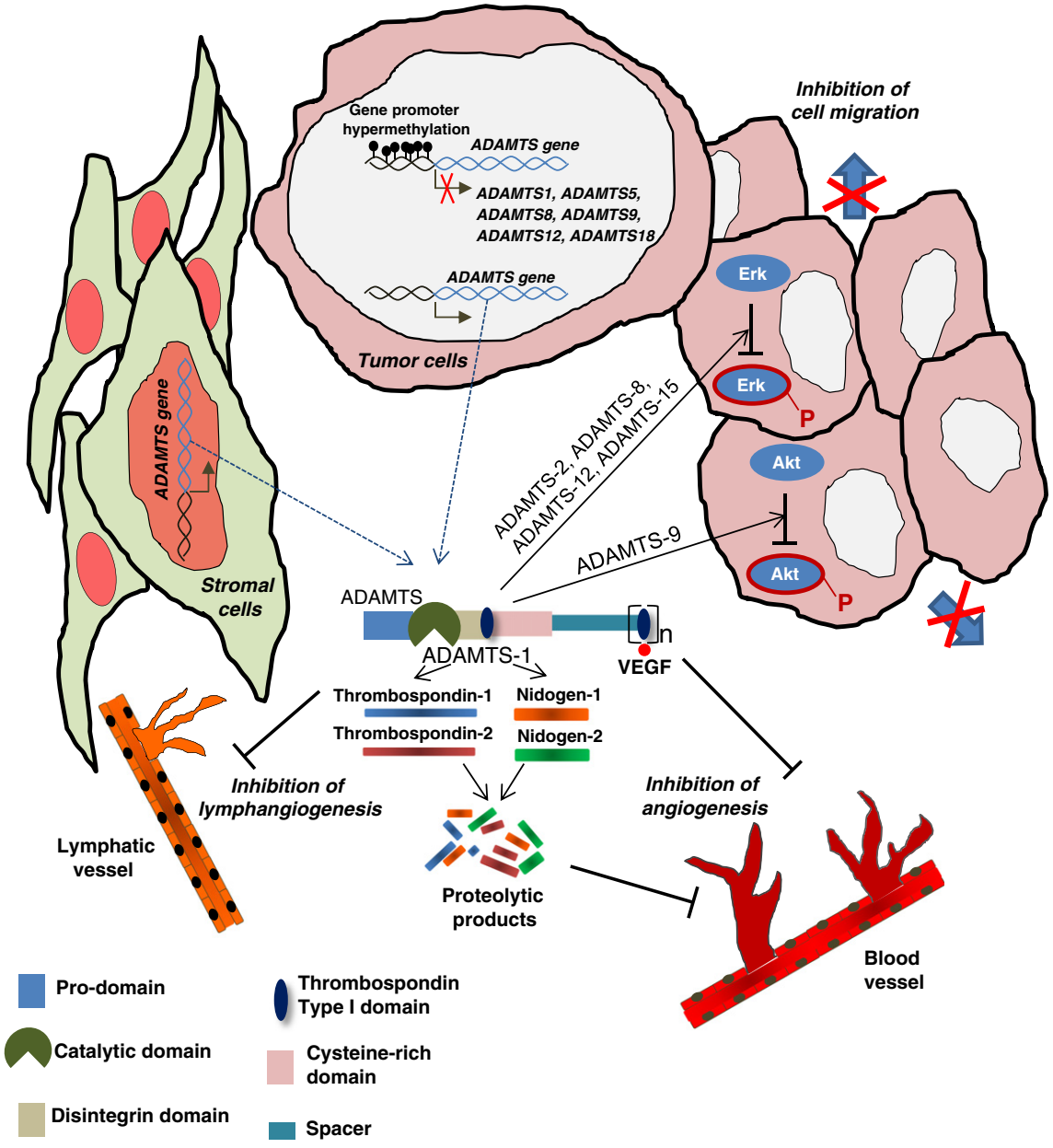

Fig. 1. Schematic representation of the antitumor effects mediated by ADAMTS metalloproteases. These effects have been mainly described for ADAMTS-1. ADAMTSs can be produced by stromal or tumor cells, and exert antitumor properties inhibiting angiogenic or lymphangiogenic processes, or blocking tumor-promoting signaling pathways in tumor cells. These effects may be both dependent (degradation of extracellular components such as thrombospondin-1 and -2, and nidogen-1 and -2), or independent (VEGF sequestration) of the catalytic activity. Several *ADAMTS* genes are silenced through epigenetic modification in tumors from different origins. Structural domains of ADAMTSs are indicated.

spacer-2 region between the fourth and fifth TS1 motifs in ADAMTS-7 and ADAMTS-12; the GON-1 domain at the carboxy-terminal end of ADAMTS-9 and ADAMTS-20; the PLAC domain in ADAMTS-2, -3, -6, -7, -10, -12, -14, -15, -16, -17 and -19; and the CUB domains in ADAMTS-13 [5]. These structural domains could influence protease activity and led to consider ADAMTSs as a new family of secreted metalloproteases [6–8]. Phylogenetic analysis of the 19 human ADAMTSs allowed their classification into different subfamilies of closely related members [4,5].

Functional characterization of different ADAMTSs revealed that they were involved in key biological processes. For instance, ADAMTS-2, ADAMTS-3 and

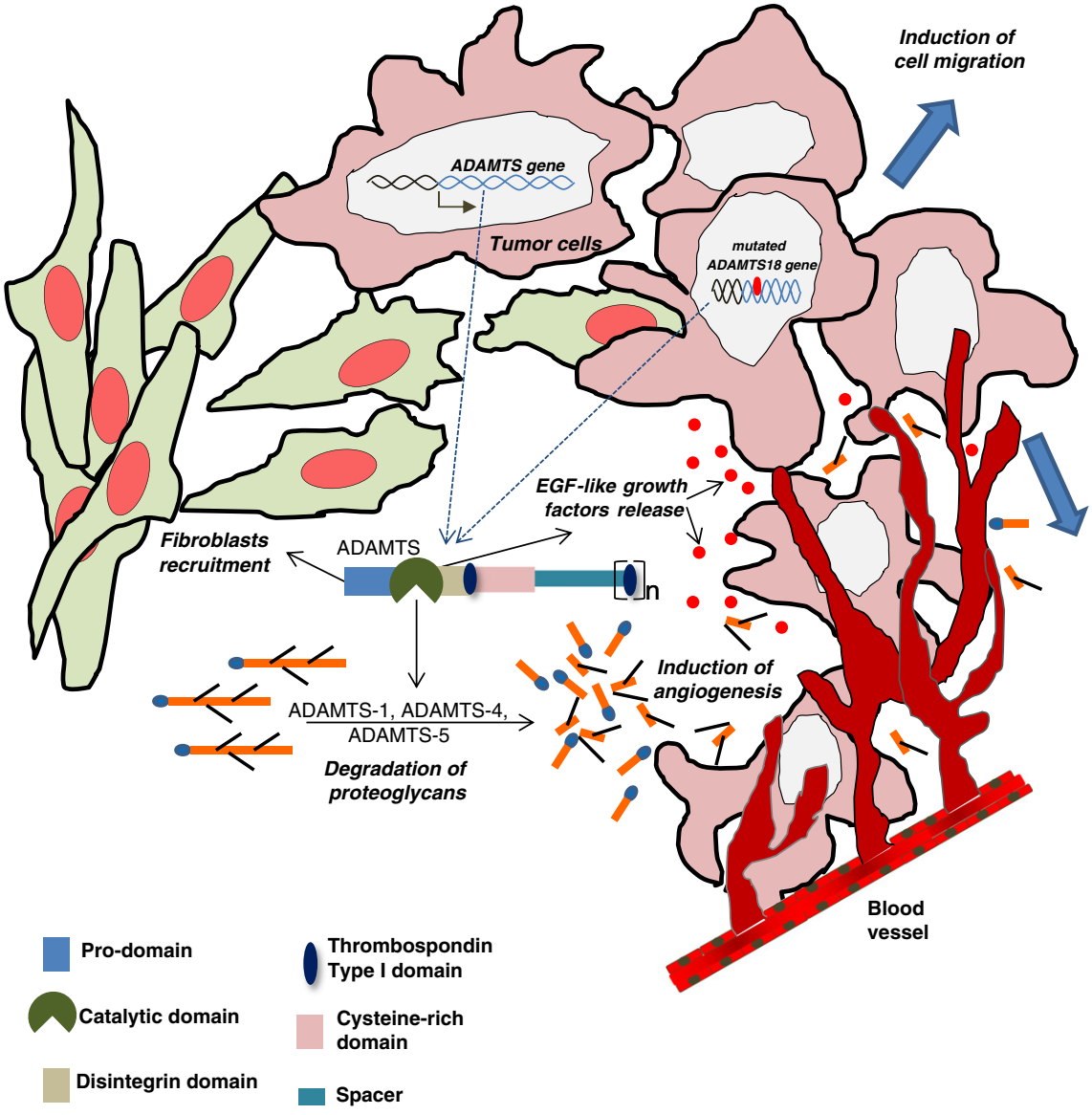

Fig. 2. Schematic representation of the protumor effects mediated by ADAMTS metalloproteases. ADAMTSs may cleave or induce the release or activation of pro-angiogenic factors (HB-EGF, amphiregulin, IGFBP2), and digest extracellular matrix components (proteoglycans) to facilitate tumor cell spreading and metastasis. Protumor effects elicited by ADAMTSs are protease-dependent. Additionally, ADAMTSs can induce the recruitment of fibroblasts involved in tumor growth. Genetic alterations of *ADAMTS* genes can also induce protumor effects. Structural domains of ADAMTSs are indicated.

ADAMTS-14 are procollagen N-proteinases [9–11], and loss-of-function mutations in *ADAMTS2* cause Ehlers–Danlos syndrome VIIC, a connective tissue disorder characterized by severe skin fragility [12]. ADAMTS-4 and ADAMTS-5/11 are responsible for aggrecan degradation in osteoarthritic diseases [13,14]. *ADAMTS10* mutations underlie Weill–Marchesani syndrome [15], an acromelic dysplasia associated with short stature, eye abnormalities, brachydactyly and joint stiffness. A Weill–Marchesani-like syndrome is caused by mutations in the *ADAMTS17* gene [16]. In this case, affected individuals exhibit some of the symptoms of Weill–Marchesani syndrome, but they show no signs of brachydactyly or decreased joint flexibility. ADAMTS-13 is the enzyme responsible for the cleavage of large multimers of von Willebrand factor [17], and defects in the gene encoding this metalloprotease cause thrombotic thrombocytopenic purpura, a life-threatening hematologic disease characterized by the inability of blood to clot normally [18]. *Adamts20* is mutated in belted mice, which lack pigment in the belt-like region of the trunk as a result of a

defect in melanocyte development [19]. These examples illustrate the functional relevance of ADAMTSs not only in normal biological events but also in pathological conditions.

ADAMTSs have also been associated with cancer as their expression was found to be altered in tumors from different origins, similarly to what happened with the founder member of the family ADAMTS-1 in colon cancer cachexia [8]. However a growing number of studies have also highlighted the tumor-protective effects of ADAMTSs [20,21]. Different mechanisms have been linked to the protumor or antitumor activities of these metalloproteases, including regulation of angiogenesis, cell proliferation, adhesion and migration, as well as degradation or interaction with extracellular matrix components. Moreover, several *ADAMTS* genes have been found mutated or epigenetically silenced in specific tumors. In this review, we summarize the tumor suppressive effects and the oncogenic functions elicited by ADAMTS metalloproteases.

Tumor-protective effects of ADAMTSs

Following its identification in a mouse colon cancer cachexia tumor cell line [1], ADAMTS-1 or METH-1 was described as an angio-inhibitory enzyme due to its capacity to restrain endothelial cell proliferation. The carboxy-terminal half region of the enzyme, comprising the TS1 motifs, is responsible for this inhibitory effect, which occurs by sequestration of VEGF165 [22,23]. The ADAMTS-1 catalytic domain is not directly required to elicit anti-angiogenic effects in different tumor cell lines [24]. However, ADAMTS-1 proteolytic activity mediates the release of angio-inhibitory polypeptides from thrombospondin-1 and -2 [25]. Thus, fragments generated through proteolytic cleavage of thrombospondin-1 by ADAMTS-1 suppress vascularization of liver metastasis [26]. Nonetheless, overexpression of thrombospondin-1 does not affect vascularization of lung metastasis, an organ that exhibits low ADAMTS-1 activity [26]. In addition, it has also been recently shown that ADAMTS-1 is responsible for the degradation of nidogen-1 and -2 in a mouse model of breast cancer, which contributes to enhance the anti-angiogenic response by the metalloprotease [27]. Angio-inhibitory effects, using cell-based assays or animal models, have also been associated with other ADAMTSs including ADAMTS-2 [28], ADAMTS-4 [29], ADAMTS-5 [30,31], ADAMTS-8 or METH-2 [22,32], ADAMTS-9 [33,34], and ADAMTS-12 [35]. Nevertheless, different mechanisms seem to underlie these angio-inhibitory capacities. For instance and similarly to ADAMTS-1, ADAMTS-2 [28], ADAMTS-5 [30,31] and ADAMTS-12 [36] do not require their catalytic activity to block angiogenesis, an effect most likely related to the TS1 motifs. By contrast, proteolytic activity of ADAMTS-9 [34] and ADAMTS-15 [37] are essential to promote angio-inhibitory capacity. Contrary to ADAMTS-1, ADAMTS-9 does not cleave thrombospondins 1 and 2 and does not bind VEGF165 [34]. However, ADAMTS-4 is also able to capture and inactivate VEGF and, additionally, to inhibit phosphorylation of VEGF receptor 2 (VEGFR2) [29]. An early report by Iruela-Arispe et al. showed that ADAMTS-1 can also inactivate VEGFR2 in human aortic endothelial cells [38]. More recently it has also been found that ADAMTS-1 is able to block VEGFR3 phosphorylation in human dermal lymphatic microvascular endothelial cells HMVEC-dLy, leading to an inhibition of lymphangiogenesis [39]. These findings point out the importance of ADAMTSs as modulators of both angiogenic and lymphangiogenic responses (Fig. 1).

Different ADAMTSs may exert their antitumor effects through the blocking of Erk phosphorylation, a signaling pathway usually increased in tumor cells [40]. This inhibitory effect was initially described for the *Xenopus* ortholog of ADAMTS-1 [41]. Furthermore, and consistent with ADAMTS angio-inhibitory capacities, it has been shown that ADAMTS-2 decreases Erk phosphorylation levels in HUVEC cells but not in fibroblasts [28], and that ADAMTS-12 inhibits Erk phosphorylation and tubulogenesis in MDCK cells upon stimulation with hepatocyte growth factor [36]. Abrogation of Erk phosphorylation has also been linked to ectopic expression of ADAMTS-15 in colon cancer cells [42], and ADAMTS-8 in epithelial and esophageal carcinoma cells [43]. However, levels of Erk phosphorylation remain unaltered when ADAMTS-9 is exogenously expressed in gastric cancer cells, whereas levels of phosphorylated Akt and phospho-mTOR are reduced [44]. These findings indicate that ADAMTSs may modulate different cancer-related signaling pathways. Functional consequences of the ectopic expression of these ADAMTSs were analyzed using different cell-based models, including wound-healing, cell proliferation, and tumor formation in nude mice. For instance, ADAMTS-1 inhibits tumor growth when human fibrosarcoma HT-1080, human prostate cancer DU-145 and Chinese hamster ovary CHO-K1 cells are subcutaneously inoculated in mice [24]. Likewise, the presence of ADAMTS-15 in mammary cancer cells decreases cell migration on extracellular matrix components such as fibronectin or laminin [37]. Conversely, knock-down of ADAMTS-1, ADAMTS-9 and ADAMTS-15 increases the tumorigenic potential of breast, gastric and colon cancer cells [42,44,45]. Moreover, reduction of ADAMTS-9 expression using RNA interference in a nontumorigenic HONE1 epithelial cell line causes the restoration of a tumorigenic phenotype [33]. In this regard, ADAMTS-13 deficiency has been related to neoplasia-associated microangiopathic hemolytic anemia in colon cancer patients [46]. These studies indicate that loss-of-function of ADAMTS metalloproteases may contribute to increase tumor cell malignancy.

Antitumor effects of ADAMTSs can also be inferred by the fact that several *ADAMTS* genes have been found epigenetically silenced in tumors from different origins (Fig. 1). Thus, *ADAMTS1* shows high frequency of promoter methylation in pancreatic, colorectal and lung cancer [47–49]; *ADAMTS5* in colorectal cancer [50]; *ADAMTS8* in brain; thyroid, lung, nasopharyngeal, esophageal, gastric and colorectal cancers [32,43,51,52]; *ADAMTS9* in esophageal, nasopharyngeal, gastric, colorectal and pancreatic cancers [33,44,53,54], as well as in multiple myeloma [55]; *ADAMTS12* in colon cancer [56], and *ADAMT18* in gastric, colorectal, pancreatic, esophageal and nasopharyngeal carcinomas [57,58]. It is noteworthy that some ADAMTSs can be also found expressed in stromal cells but not in cancer cells [59]. This is the case of ADAMTS-12, which has been detected in cancer-associated fibroblasts but not in tumor cells in colon carcinoma samples [56]. The presence of this metalloprotease has been related to a stromal response aimed to control tumor progression, thereby ADAMTS-12 has emerged as a potential marker of good prognosis in this type of tumor [56,60]. Conversely, ADAMTS-15 is detected in colon cancer cells rather than in stromal cells, and its expression inversely correlates with the histopathological grade of tumors [42]. Notably, *ADAMTS15* gene is not epigenetically silenced, but it has been found frequently mutated not only in colon but also in pancreatic carcinomas [42,61,62]. Tumor-suppressive effects elicited by ADAMTS-15 have also been described in breast carcinomas and its detection is associated with a better clinical outcome in breast cancer patients [59,63]. Nevertheless, additional studies would be necessary to determine whether *ADAMTS* genes are frequently mutated in tumors, as well as to evaluate the functional implications of these mutations in cancer development. Data available at COSMIC database (cancer.sanger.ac.uk) suggest that indeed, distinct *ADAMTS* genes can be genetically inactivated in different types of tumors.

Protumor effects of ADAMTSs

ADAMTS-1 is the best characterized tumor-promoting ADAMTS metalloprotease. In fact, shortly after its discovery, ADAMTS-1 was found overexpressed in pancreatic cancer and proposed to be involved in tumor progression facilitating local invasion and lymph node metastasis [64]. Also, recent studies showed that *ADAMTS1* overexpression in a fibrosarcoma model increased tumor growth rate that was angiogenesis independent [65]. Furthermore, in conflict with the angio-inhibitory effect derived from the proteolytic cleavage of thrombospondin-1, the enzymatic activity of ADAMTS-1 is required to enhance angiogenesis and to promote pulmonary metastasis of both Lewis lung carcinoma and TA3 murine mammary carcinoma cells [66]. These processes are accompanied by the shedding of heparin-binding epidermal growth factor (HB-EGF) and amphiregulin, and the activation of epidermal growth factor receptor (EGFR) (Fig. 2). By contrast, the overexpression of a catalytically inactive ADAMTS-1 impedes these events, which strongly suggests a prometastatic role for this metalloprotease mediated by its proteolytic activity [66]. Elevated ADAMTS-1 expression has also been associated with high risk of bone metastasis in breast cancer patients [67,68].

Further studies using animal models have provided additional support to the proposed protumor effects of ADAMTS-1 in mammary carcinomas. In fact, both growth of mammary tumors and lung metastasis are reduced in *Adamts1*-deficient mice compared to wild-type mice using the MMTV-PyMT mouse mammary tumor model [69]. Notably, a high accumulation of cleaved versican is found in the tumor microenvironment in wild-type mice, but not in knock-out mice. Thus, it has been proposed that ADAMTS-1 could facilitate the spreading of tumor cells through the degradation of versican, a predictor of metastatic relapse in human breast cancer [70]. In addition, it has been reported that metastasis of breast cancer cells can be promoted by the ADAMTS-1 mediated cleavage of the extracellular matrix component semaphorin 3C [71]. ADAMTS-1 catalytic activity is also involved in a stromal response to recruit fibroblasts, thus promoting lung cancer tumor growth [8]. Moreover, breast tissue-associated fibroblasts also contribute to promote cancer cell invasion through up-regulation of ADAMTS-1 levels (Fig. 2) [72]. Furthermore, ADAMTS-1 may associate its proteolytic action with MMP-1 activity to promote osteolytic bone metastasis by shedding of different membrane-bound epithelial growth factor (EGF-like) factors [68]. Recently, insulin-like growth factor binding protein-2 (IGFBP2) has also been identified as a target of ADAMTS-1, and a high expression of the metalloprotease correlates with higher levels of cleaved IGFBP2 in glioblastoma [27]. It is noteworthy that ADAMTS-1 proteolytic activity can be increased by the interaction with fibulin-1, a secreted glycoprotein that also displays protumor and antitumor effects [73,74]. In turn, fibulin-1 can interact with different extracellular matrix components including known ADAMTS-1 substrates such as nidogen-1 and versican [75]. Consequently, fibulin-1 could play a crucial role in the balance of protumor effects versus tumor-protective functions displayed by ADAMTS-1. In any case, these studies support the influence of ADAMTS-1 catalytic activity in the remodeling of the tumor microenvironment to facilitate the spreading of tumor cells in different types of cancer [76].

ADAMTS-4 and ADAMTS-5 are proteoglycanases that can contribute to increase the invasive potential of glioblastoma cancer cells through the degradation of brevican (Fig. 2), a highly expressed proteoglycan in this type of malignant brain tumor [77,78]. Notably,

a mutant form of brevican resistant to ADAMTS cleavage does not enhance glioma cell invasion, highlighting the importance of the proteolytic processing of brevican mediated by ADAMTS in the invasiveness of tumor cells [79]. Furthermore, the N-terminal cleavage product of brevican binds to fibronectin and increases glioma cell dispersion [80]. The presence of high levels of ADAMTS-4 has also been linked to an increase of tumorigenic potential in head and neck squamous cell carcinomas [81], and in Ewing's sarcomas, where this metalloprotease has emerged as a potential tumor marker [82]. It is noteworthy that ADAMTS-4 undergoes an autocatalytic processing similar to that described for ADAMTS-1, which affects balance between protumorigenic and antitumorigenic functions of this metalloprotease [83]. Thus, an N-terminal 53-kDa catalytically active isoform of the enzyme promotes B16 melanoma angiogenesis in mice. In clear contrast, the catalytically inactive full-length protein or different truncated fragments containing C-terminal ancillary domains impede melanoma cell growth and angiogenesis process. In reference to ADAMTS-5, this is the main aggrecanase detected in laryngeal carcinomas and its catalytic activity may contribute to facilitate dissemination of tumor cells [84].

Protumor activities have also been reported for ADAMTS-12. Thus, this metalloprotease can potentiate trophoblast invasion by a mechanism involving $\alpha_v\beta_3$ integrin [85]. In a recent report, it has also been shown that ADAMTS-12 potentiates migration of MCF-7 breast cancer cells when is expressed in absence of fibulin-2 [86]. Interestingly, ADAMTS-12 is able to interact with fibulin-2 and a concomitant expression of both extracellular proteins considerably reduces protumor capacities of breast tumor cells [86]. Furthermore, combined high expression of both ADAMTS-12 and fibulin-2 correlates with the best prognosis in breast cancer patients. Fibulin-2, similarly to fibulin-1, may act as a protumor or antitumor glycoprotein [74,87]. For instance, fibulin-2 drives malignant lung cancer progression [88], but reduces breast cancer cell invasion [89]. Therefore, fibulin-2 could act as a modulator between tumor-promoting and anti-oncogenic roles associated to ADAMTS-12.

Other ADAMTS metalloproteases showing tumor-associated effects are ADAMTS-2, which is upregulated in osteosarcoma cells following treatment with TGF-β [90]; ADAMTS-14, which is part of an expression signature involved in the generation of experimental bone metastasis by IGR-CaP1 human prostate cancer cells [91]; and ADAMTS-18, which is the ADAMTS metalloprotease most frequently mutated in melanoma and some of these mutations promote cancer growth and metastasis of melanoma cells [92]. Overall, these findings illustrate the very different and complex protumor actions that may be elicited by the ADAMTS metalloproteases.

Conclusions and future directions

Alteration of the tumor microenvironment is essential to promote cancer growth and metastasis. Secreted or membrane-associated metalloprotease activities have been traditionally associated with an increase of the tumorigenic potential of tumor cells. However, a growing number of studies support the tumor-suppressive role of different metalloproteases [93]. In relation to ADAMTSs, these enzymes can be secreted by tumor or stromal cells and then modify the primary tumor microenvironment by proteolytic-dependent or independent mechanisms. Thus, it could be speculated that pro-tumor functions or anti-oncogenic properties elicited by the ADAMTSs may depend on the substrates or interacting-partners present in the cell microenvironment. A future challenge would be to differentiate the nature of ADAMTS-mediated interactions and processing events of regulatory growth factors or extracellular matrix components that potentiate tumor growth from those that suppress metastasis. Additionally, a more profound knowledge of the molecular mechanisms regulated by ADAMTSs would contribute to better understand the real impact of these metalloproteases in tumorigenesis. Hopefully, clarification of these processes will help to introduce more specific and less toxic personalized antitumor therapies.

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